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First insight into *Araucaria araucana* (Molina) K. Koch under its southernmost European growing condition: a proposed descriptor list for morphological characterization

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Abstract: *Araucaria araucana* is a south American endemic Conifer of conservation concern. After its introduction to Europe, this species has been often planted for ornamental purposes in parks and gardens, where its unusual appearance was admired. After the mid-1970s this tree received increasing attention from the nursery growers of Pistoia (Tuscany) and became of considerable economic importance. However, being one of the iconic threatened trees listed in CITES Appendix I, the international trade of these species is rigorously regulated. This study was aimed at developing a first morphological descriptor list for further phenotyping of *in loco* produced plant material. A first step of the research focused on the description and a better understanding of *A. araucana* phenological phases inferred from Mediterranean climate conditions. The second phase regarded the analysis of observed or measured morphological characteristics of the tree, branches, scales, inflorescences, fruits and seeds observed on a subset of 4 selected putative populations over a 3-year period of vegetative growth. The results allowed to select 39 most discriminant descriptors, which are presented together with their range of variability and classes. The achieved descriptor list represents a suitable tool for the selection of genotypes and for the breeding of *A. araucana*.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

Araucaria araucana (Molina) K. Koch, commonly known as 'monkey puzzle tree' or 'pehuèn', is an endangered conifer species native to south-central Chile and south-western Argentina, where it has a relatively limited distribution, split between the main area spanning both sides of the Andes and two other disjunct small subpopulations in the Coastal Cordillera of Chile (Donoso, 1993, 2006; Donoso *et al.,* 2008; Drake *et al.,* 2009). The present distribution is a remnant of a more extensive former distribution, which has been severely diminished by logging, human-set

fires and land clearance since European colonization in the mid-19th century (Veblen, 1982; Burns, 1993; Rechene, 2000). In particular, the intense human seed collecting and animal grazing have led to a lack of natural reproduction by seed, and when any regeneration occurs it is principally asexual with tree sprouting from roots (Schilling and Donoso, 1976; Gallo *et al.,* 2004). Since 1976, this species has been protected in Chile under the status of a Chilean National Monument and, since 1997, it is also protected internationally under the Convention on International Trade in Endangered Species of Wild Fauna and Flores (CITES) (Farjon and Page, 1999; Herrmann, 2006).

Araucaria araucana was first introduced in England by the Scottish naturalist Archibald Menzies in 1795. Its unusually straight cylindrical bole and whorled branches as well as its 10-15 cm thick tortoise-shell-like bark made it internationally popular as an ornamental plant. The following decades saw a rapid spread of this impressively large and long-lived conifer throughout all the European continent. It was introduced in Italy from Paris in 1822 and the first *A. araucana* tree was planted in the garden of the Marquis Pucci's favourite property in Florence, Tuscany. This Italian region represents the southernmost limit of its distribution area. Thanks to its perfect adaptability to the Tuscan soil and climate conditions, this majestic slow-growing tree was included in the important ornamental horticulture district of Pistoia (NW from Florence) starting from the II post-world war period. Thereafter, there was a notable and rapid increase of *A. araucaria* commercial propagation in the Pistoia district in line with the steadily-growing demand. This tree became of considerable economic importance and the period between the 1970s till the early 2003 saw the maximum expansion of commercialization rate and tree planting in private properties and public gardens. Due to its constantly declining distribution, together with its slow growth and its limited dispersal ability, in 2003, according to the Regulation (CE) n. 1497, the listing of this species was transferred from Appendix II to Appendix I of CITES (http://www.cites.org/eng/app/appendices.html; valid from October 4th, 2017), which strictly regulates the trade in its timber and seeds, and listed in the 2008 IUCN Red List of Threatened Species (http://www.iucnredlist.org; March 2017) as an endangered species currently on risk of extinction. As a consequence, plant nurseries had to adopt a mandatory stock register of alive and dead specimen, where both entries and exits (including origin, quantity, causes of death, etc.) had to be specified. A progressive and ongoing reduction of monkey puzzle tree propagation in the Pistoia district has resulted from increased regulation, complex management, high risk of penalties, increased costs for staff training, and risks from plant diseases associated with climate change. Ultimately, the number of nurseries holding and propagating *Araucaria* plants conspicuously declined during the last fifteen years.

The identification of a new Tuscan variety could offer the opportunity to disengage from the procedural constraints imposed by the CITES Convention. Hence, the production of a descriptor list for the characterization of Tuscan selected germplasm is a necessary first step towards the definition of genetic diversity based on morphological variation and for varietal identification in *Araucaria*. The descriptor list might represent the first attempt at achieving an unified documentation system thereby enabling through a standardized format an easier exchange of information between researchers and collection curators. Although there is a high demand for new descriptor lists to be developed for many forest conifer species, up to our knowledge there is only an UPOV descriptor list available for *Picea abies* L. (https://www.upov.int/test_guidelines/en/fulltext_tgdocs.jsp?q=Picea; copyright © 2011, UPOV).

Despite the conservation interest in this species, little is known of its phenotypic and genetic variation. The genetic diversity of monkey puzzle between Andean and coastal Chilean populations has been investigated in previous studies by Delmastro and Donoso (1980) and Rafii and Dodd (1998). More recently, advanced biotechnologies, such as RAPDs, Isozymes, microsatellite and RFLP analysis, were used to characterize genetic heterogeneity within and among some South American populations (Bekessy *et al.,* 2002; Ruiz *et al.,* 2007; Marchelli *et al.,* 2010; Martín *et al.*, 2012). However, no reports were found in the world literature on morphological traits.

This study was part of the CARAVIV project 'Characterization of *Araucaria araucana* germplasm selected by the nursery industries of the Pistoia's district for commercial development', supported by the Ministero delle Politiche Agricole Alimentari e Forestali (MiPAAF-OIGA), aimed at contributing to a better understanding of *Araucaria* growing and to enhance the commercial exploitation of local genetic resources. This paper provides a brief account of *Araucaria araucana* phenological phases under Italian climatic conditions and is focused on the development of a first descriptor lists in order to

characterize *in loco* produced plant material and make information available to other growers in a systematic and unambiguous form.

2. Materials and Methods

Plant material

The plant material used in this study was the *A. araucana* germplasm available in the Pistoia' nursery district, covering an area of approx. 965 sq km, ranging from 50 m to 550 m above sea level, located in northern Tuscany.

Phenology

Throughout three-year growing cycles (2015-2017), the phenological phases (onset of flowering, full bloom, fertilization, fruit ripening and seed production) of *A. araucana* trees belonging to 8 putative populations were observed every two weeks from March to June (during the flower maturation), and monthly in the rest of the year. Taking into account the peculiar structure of the reproductive buds of this species, we decided to consider as onset of flowering the inflorescence appearance and as full bloom the inflorescence maturity, i.e. the pollination phase. Four out of the selected populations belong to Pistoia's hinterland in a plain area (43°53' N; 10°55' E; 60 m a.s.l.), while the remaining four are located in high hills (44°0' N; 10°52' E; 550 m a.s.l.). The number of plants for each population ranged from 10 to more than 200, varying in age, gender and sexual maturity.

Morphological descriptor list

Twenty/twenty-five-year-old specimens belonging to a subset of 4 putative populations were randomly defined in order to perform a morphological description. The considered populations were derived from seeds of different origin (unknown, Dutch fair, Spanish fair, local selected progeny) and grown in three private nurseries, located very close to each other in a plain area under the same organic regime. Local selected progeny refers to seeds collected from a couple of old trees located in Villa Lodolo (S. Marcello Pistoiese, 44°03' N; 10°47' E; 623 m a.s.l.). These trees were introduced from Argentina in 1920 and represent the main genetic source of *A. araucana* local germplasm. Climatic data, i.e. atmospheric pressure (AP), medium (AVG T), maximum (MAX T) and minimum (MIN T) air temperatures, relative air humidity (RH), wind run (WR), global horizontal irradiation (GHI), rainfall (RAIN), evaporation (EV), for the same area were collected monthly from January 2015 to December 2017 (Table 1).

Minimum, maximum, average, standard deviation and coefficient of variability (%) values of each morphometric character were calculated for the whole set of plants by using one-way analysis of variance (ANOVA). This in turn led to the definition of classes for all the measured traits by subdividing the total range into intervals 2 times the standard deviation, i.e. whether above or below the average value of each parameter as described in Bassi (2003). Statistical analysis was performed using SPSS 20 software (Chicago, IL, USA).

Table 1 - Seasonal averages (2015-2017) of main meteorological data collected in Pistoia's nursery area[a]

Year	Season	AP (mbar)	AVG T (°C)	MAX T (°C)	MIN T (°C)	RH (%)	WR (km)	TSI (kWh/m²)	RAIN (mm)	EV (mm)
2015	Winter	1009.33	8.33	14.73	3	68.33	86.7	1.53	2.7	1.6
	Spring	1011	18.7	26.67	11.03	62.33	73.37	3.93	1.73	4.9
	Summer	1009	24.77	33.13	16.87	57	69.6	4.17	1.23	6.5
	Fall	1016.33	11.5	18.07	6.97	78.33	33.73	1.17	3.67	1.07
2016	Winter	1007.33	9.1	14.9	4.47	74	77.6	1.37	5.9	1.4
	Spring	1007	16.83	25.2	9.83	65	77.9	3.7	2	5
	Summer	1010.67	24.2	33.03	16.37	57.67	66.9	4.2	1.57	6.5
	Fall	1014.67	10.43	18.3	5.1	78	24	1.3	3.57	1.03
2017	Winter	1012.33	8.47	15.77	2.43	67.33	66.4	1.67	3.53	1.77
	Spring	1009.67	19.5	27.7	11.53	60	76.03	4.07	1.4	6.3
	Summer	1009.33	23.67	32.47	15.43	57	65.6	4.03	1.7	6.43
	Fall	1011.33	9.93	18	4.3	76	28.3	1.27	5.13	1.1

AP= atmospheric pressure; AVG T= medium air temperature; MAX T= maximum air temperature; MIN T= minimum air temperature; RH= relative air humidity; WR= wind run; GHI= global horizontal irradiation; RAIN= rainfall; EV= evaporation.

[a] Processed from Ce.Spe.Vi database, Pistoia (http://www.cespevi.it/meteo.htm; Paolo Marzialetti © 1996/2018 Ce.Spe.Vi. - Pistoia).

3. Results

Phenology

The results of our observations throughout the growing cycles of male and female *A. araucana* trees in the Pistoia district are shown in figure 1. The main collected phenological data (flowering onset, flower maturation, fertilization, fruit ripening and seed production) were compared with those found in previous published surveys on native Andean populations (Table 2).

Morphological descriptor list

Starting from field observations over a three-year growing cycle, a total number of 39 descriptors were developed for further germplasm phenotyping, divided into 6 sections: 1) tree, 2) branches, 3) leaves, 4) male inflorescences, 5) female strobiles, 6) seeds and productivity. These descriptors apply to twenty/twenty-five-year-old trees (i.e. approx. the age of first fruit bearing) grown in nurseries as ornamental plants. The descriptor list has been enriched by images and drawings for a better understanding

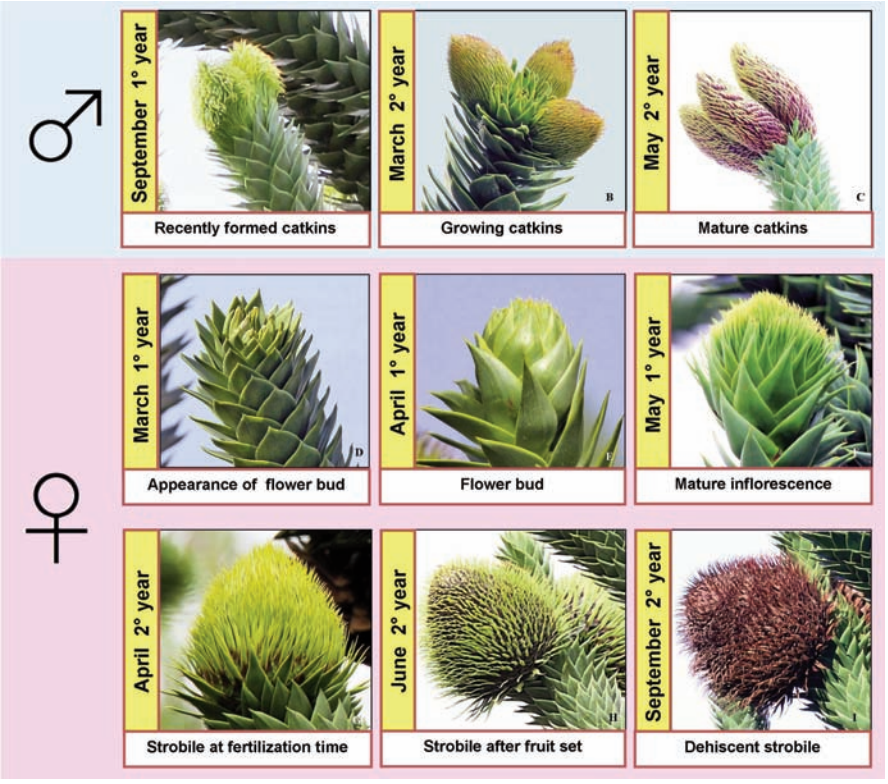

Fig. 1 - *Araucaria araucana* male (♂) and female (♀) phenological stages under Tuscan climate conditions.

Table 2 - Differences between the maturation stages of female and male inflorescences and seed production in *Araucaria araucana* populations in their origin area and in Italy

Phenological phases	Chile/Argentine[a]		Italy	
	♀	♂	♀	♂
Flowering onset	Nov. I	Aug. I / Sep. I	Mar. I	Jul. /Aug. I
Maturation	Dec. I	Dec. I	May I	May II
Fertilization	Jan. II	-	Apr. II	-
Seed production	Dec. II / Feb. III	-	Sep. / Oct. II	-

I = first year; II second year; III = third year.

[a] Tortorelli, 1956; Montaldo, 1974; Donoso and Cabello, 1978; Rodriguez *et al.*, 1983; Marticorena, 1995.

of the less familiar and discernible traits.

Descriptor list EXAMPLES [a]

TREE

1. Size (height) (m)
□ Low <3
□ Medium 3-8
□ High >8

2. Trunk diameter, 1m from collar (mm)
□ Small <70
□ Medium 70-110
□ Large >110

3. Shape of canopy
□ Globose (3a)
□ Elliptic (3b)
□ Pyramidal (3c)
□ Columnar (3d)

3a 3b 3c 3d

4. Density of canopy
□ Sparse (4a)
□ Medium (4b)
□ Dense (4c)

4a 4b 4c

5. Distance among scaffold branches (mm)
□ Low <250
□ Medium 250-400
□ High >400

6. Colour of bark
□ Light grey (6a)
□ Greyish-green (6b)
□ Greyish-brown (6c)

6a 6b 6c

7. Density of scales on the trunk
□ Sparse
□ Medium
□ Dense

8. Insertion angle of the scales on the trunk
□ Angle ≤ 30°(8a)
□ Angle >30° (8b)
□ Angle variable (8c)

8a 8b 8c

9. Homogeneity of the trunk scales
□ Homogeneous shape (9a)
□ Inhomogeneous shape (9b)

9a 9b

10. Lenght of the trunk scales (mm)
□ Short <35
□ Medium 35-50
□ Long >50

11. Widht of the trunk scales (mm)
□ Narrow <15
□ Medium 15-20
□ Large >20

BRANCHES

12. Length of the first internode on the primary branch (mm)
□ Short <25
□ Medium 25-50
□ Long >50

13. Length of the median internode on the primary branch (mm)
□ Short <15
□ Medium 15-25
□ Long >25

14. Density of branching: number of secondary branches on primary branch
□ Sparse <8
□ Medium 8-14
□ Dense >14

15. Density of scales on the secondary branch
□ Sparse (15a)
□ Medium (15b)
□ Dense (15c)

15a 15b 15c

16. Average number of branches per scaffold

17. Apparent diameter of the primary branch including scales (mm)

Wide section area	Narrow section area
□ <8	□ <6
□ 8-12	□ 6-9
□ >12	□ >9

18. Apparent diameter of the secondary branch including scales (mm)

Wide section area	Narrow section area
□ <6	□ <4
□ 6-8	□ 4-6
□ >8	□ >6

19. Uniformity of the apparent diameter on the primary branch
□ Uniform
□ Difform
□ Very difform

20. Uniformity of the apparent diameter on the secondary branch
□ Uniform (20a)
□ Difform (20b)
□ Very difform (20c)

20a 20b 20c

21. Insertion angle of the scales on the primary branch
- ❑ Angle ≤ 45° (21a)
- ❑ Angle >45° (21b)

21a 21b

LEAVES (scales)

22. Maximum length including mucron (mm)
- ❑ Short <28
- ❑ Medium 28-40
- ❑ Long >40

23. Maximum width (mm)
- ❑ Narrow <10
- ❑ Medium 10-16
- ❑ Wide >16

MALE INFLORESCENCES (catkins at the mature stage)

24. Catkin lenght (mm)
- ❑ Short <81
- ❑ Medium 81-123
- ❑ Long >123

25. Catkin thickness (mm)
- ❑ Thin <40
- ❑ Medium 40-54
- ❑ Thick >54

26. Catkin bending
- ❑ Attenuated (26a)
- ❑ Medium (26b)
- ❑ Marked (26c)

26a 26b 26c

FEMALE STROBILES (pine-cones at harvest)

28. Pine-cone max. diameter (mm)
- ❑ Small <170
- ❑ Medium 170-200
- ❑ Large >200

29. Maximum length of the scale including appendix (mm)
- ❑ Short < 36
- ❑ Medium 36-44
- ❑ Long >44

30. Maximum width of the scale (mm)
- ❑ Narrow <7
- ❑ Medium 7-9
- ❑ Wide >9

SEEDS and PRODUCTIVITY

31. Number of catkins

32. Number of female cones

33. Total number of seeds

34. Number of fertile seeds

35. Color of fertile seeds [b]
- ❑ Reddish brown
- ❑ Greenish brown
- ❑ Dark brown

36. Length of the seed appendix excluded (mm) [b]
- ❑ Short <37.8
- ❑ Medium 37.8-45.6
- ❑ Long >45.6

37. Width of the seed (mm) [b]
- ❑ Narrow <12.3
- ❑ Medium 12.3-16.5
- ❑ Wide >16.5

38. Length of the appendix [b]
- ❑ Short <33.3
- ❑ Medium 33.3-48.3
- ❑ Long >48.3

39. Prevailing shape of the seed
- ❑ Elongated (39a)
- ❑ Oblong (39b)
- ❑ Obconical (39c)

39a 39b 39c

[a] Araucaria draws have been taken and modified from the website http://www.eryprihananto.com (© Ery Prihananto, Indonesia).

[b] Mean of 25 seeds.

4. Discussion and Conclusions

Very few studies have examined botanical aspects in *A. araucaria* following individual plants over their lifetimes, and all are rather dated and referred to plants grown under South America climatic conditions (Montaldo, 1974; Donoso and Cabello, 1978; Hoffman, 1982; Rodríguez *et al.,* 1983). No data concerning the phenology and growing of this species under both European and Italian environmental conditions have been reported elsewhere, except for a very old contribution to the understanding of cytology and sexual reproduction in *A. araucana* plants grown in Northern France (Favre-Duchartre, 1960). Søndergaard (2003) reported about new introductions of monkey puzzle to Scandinavia and the West coast of Norway, while Kubus *et al.* (2014) evaluated hardiness of *A. araucana* trees grown in open ground in Poland. However only data on annual shoot growth, tree height and degree of frost damages were given. On the other hand, phenological observations are some of the most sensitive data in identifying how plant species respond to regional climate conditions and to climatic changes.

Like all Gymnosperm, monkey puzzle has extremely simple flowers without any ornamental value. The male and female reproductive structures are carried by 'cones' and are as a rule separated, in

fact monkey puzzle is usually dioecious (Martinez, 1957; Bekessey *et al.,* 2002). Nevertheless, although being basically female, 4 out of 15 flowering trees were found to produce both male and female cones during recording in southwestern of Norway; moreover, on completely isolated male and female trees, a couple of cones of the other sex were observed in some years in the northernmost area (Søndergaard, 2003). The Author suggested a possible correlation between stress (by isolation and climatically exposed situations) and monoecious behavior in *A. araucana*. In our census, only one monoecious tree was found among all considered specimen (approx. 700 plants) of the Pistoia province. The ratio of females to males in the examined populations grown in plain area was 1:4 (on a total of approx. 25% differentiated trees) being biased towards the male sex, while a rather balanced sex ratio was observed in older (approx. forty-five-year-old) totally differentiated trees grown in high hills (Fig. 2). This finding is almost in accordance with sex occurrence reported by Søndergaard (1975), who determined half female and half male trees in a population with 76% flowering trees in the West coast of Norway. No other comparison with literature was possible for sex ratio, which has never been reported in elsewhere published data. Since the relationship between tree growth and climate appeared to be sex-dependant, in that male trees were more sensitive to land precipitation and female trees appeared more sensitive to air surface temperature during the prior period of growth (Hadad and Roig Juñent, 2016), it is probably realistic to assume that gender imbalance in favor of male or female at the beginning of sexual differentiation might vary according to different climatic environments, such as those found in Norway and Italy. On the contrary, day length did not seem to have a strong influence on the growth and development of the monkey puzzle (Søndergaard, 2003); as a matter of fact, day length dependant trees would never survive Scandinavian latitudes, since growth would begin too early and cease too late causing extensive frost damage and eventually killing the plant.

Fig. 2 - Totally differentiated *Araucaria araucana* female (A) and male (B) trees grown in Pistoia's high hills, showing strobiles and catkins formed in consecutive years.

Under the Tuscan environmental conditions, male catkins start out erect in July-August, stop growing during the winter season and then become elongated in shape, pendant and reddish-brown at maturity in May of the following year. Formed by many small scale-shaped leaflets, called microsporophyllus, they gave rise to a large quantity of pollen. In barely adult trees male caktins are found mostly in groups of 1-3 cones (Fig. 3), while older adult trees have groups of 2 up to 7-8 cones.

Fig. 3 - *Araucaria araucana* mature catkins during pollination.

First differences in the apex of potentially vegetative or female flower buds become visible at the end of March (Fig. 1D). In flower buds the apex develops into a round dome of 3-4 cm, with more elongated and less tight scales provided with long yellow-orange appendixes. Female inflorescences, having fertile scales which contain the ovules, called macrosporophylls, are distinguishable in April (Fig. 1E), grouped in light green strobiles at the extremity of the new sprouts. Greatest frequency of full bloom

and pollination was observed in May (Fig. 1C and 1F). Some differences were found in flower appearance among growing areas. Generally, flower buds developed two up to three weeks later when trees were grown at higher altitudes and experienced rigid winters. After fruit set, that occurs in April of year II (Fig. 1G), the sessile and generally solitary and immature female strobiles are erect, globular, with a symmetrical shape, and green colored. They take usually about 4-5 months to develop into ripe globular dark brown mature cones (Fig. 1I) and remain closed until the complete maturation of the seeds. Scales usually fall off at maturity in August-September of the same year, although an ongoing trend towards the postponement of fruit drop towards November-late December was observed as a consequence of the gradual rise in mean temperatures (Fig. 4). As already assessed in previous studies (Søndergaard, 2003; Sanguinetti, 2014), cones occurrence was found to vary in relation with sun exposure; more in details,

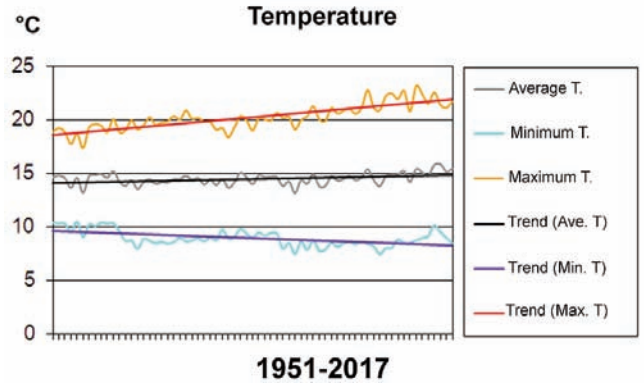

Fig. 4 - Minimum, maximum and average temperature values and trends in Pistoia (years 1951-2017) (http://www.cespevi.it/meteo.htm; Paolo Marzialetti © 1996/2018).

cones were most abundant in the South part of the crown. Almost all trees showed a marked alternate bearing; especially in males, yearly fluctuation in cone number seemed too large to be imputable only to climatic variations among years, particularly evident for rainfall (Fig. 5). Alternate bearing was found to be much more pronounced in barely sexually differentiated trees compared to plants older than fifty years, suggesting that in young plants alternate bearing represents a strategic mechanism to save nutrient reserves for significant vegetative growth.

Approx. 200-250 seeds, reddish to brown and oblong to obconical in shape, were released from each strobile; these range of seeds is fully included in those found out from the literature (150-300) (Montaldo, 1974; Donoso and Cabello, 1978; Salazar *et al.,* 2000). Negative effects of heavy rainfall on pollination and seed production have been reported (Sanguinetti *et al*., 2002).

Fructification began when plants were twenty-twenty-five-year-old, partially in agreement with reproductive organs appearance reported by Salazar *et al.* (2000) in Chilean *A. araucana* plants, while trees in Neuquén-Argentina have been reported to become sexually mature after thirty years of age, once the trunk has reached a diameter larger than 20 cm (Muñoz Ibáñez, 1984). Conversely, according to Søndergaard (2003), flowering was not initiated before the trees were forty/fifty-year-old in northern Europe and this discrepancy might be related to the altitude and latitude difference.

The ontogenetic stages presented here (Fig. 6) were in accordance with the *A. araucaria* biological

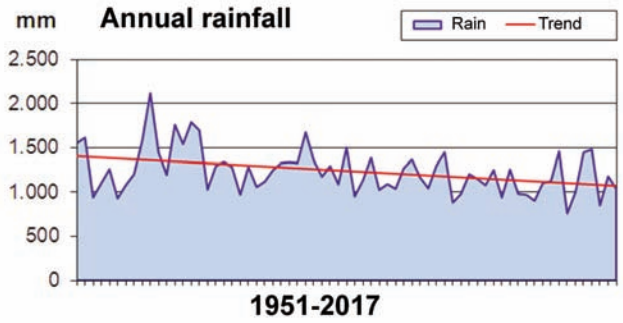

Fig. 5 - Annual rainfall values and trends in Pistoia (years 1951-2017) (http://www.cespevi.it/meteo.htm; Paolo Marzialetti © 1996/2018)

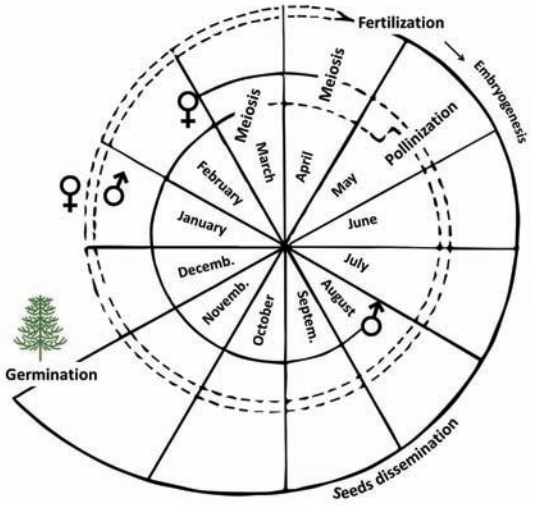

Fig. 6 - Schematic representation of the ontogenetic cycle of *Araucaria araucana*. Solid lines represent diploid phases (female and male gametogenesis) and embryogenesis after fertilization, while dashed lines represent haploid phases (ovules and pollen). (From Favre-Duchartre, 1960, modified).

cycle highlighted by Favre-Duchartre (1960) in northern France; moreover, differences in springtime temperatures between France and Norway corresponded fairly well to the differences in time (about one month) for release of pollen and seedfall at the two sites, as reported by Søndergaard (2003). On the contrary, as shown in Table 2, *A. araucana* phenology was found to differ strongly from that observed in its natural distribution in the Andes Mountains. Obviously, differences between months were principally due to the reversal of the hemispheres and consequentially of the seasons: when it is spring in Europe, it is autumn in the Andes and vice versa. However, changes seemed probably to be associated also to other factors, such as altitude, climate changes, growing conditions, etc. The effect of climate on the phenology, and in particular on the timing of reproduction, is well known for plants and extensively documented (Beebee, 1995; Stenseth and Mysterud, 2002). These studies have demonstrated that onset of reproduction in spring may have advanced by a week or two due to recent changes in climate over much of Europe, but to a much lesser extend in South America (Walther *et al.,* 2002; Stenseth *et al.,* 2002). Contrary to expectations, it was noticed that in males the appearance of the catkins takes place approximately in the same months (August-September in Chile and July-August in Italy), which, however, correspond to the end of summer in Italy and the end of winter in Chile. Moreover, in Chile the male catkins reach full maturity within 3-4 months, whereas in our environmental conditions full bloom occurred within 9-10 months. This could be explained by the fact that in Italy the further development of male inflorescence is stopped during the winter, while in Chile, where the season turns towards spring, caktins keep growing without interruption. On the other hand, maturation seems to be related to the photoperiod coinciding in both hemispheres with long days in late spring (beginning of December in Chile and end of May in Italy).

Similar considerations can be drawn for females as well. The time between female flower appearance (March in Italy and November in Chile) and maturation (end of May in Italy and beginning of December in Chile, as for males) is about 3 months in our country and 1 month in Chile. In both cases, the entry of the pollen tube into the ovary takes place within a month from the pollination. On the other hand, in our experimental conditions the true fertilization has been reported to occur after approx. 11 months (April of year II) from pollination (Favre-Duchartre, 1960), whereas timing of fecundation has never been mentioned in the literature under Andean environments. There are contradictory records about the time of seed maturation in Chile, as it is unclear if fruits release the seeds after 11-13 months (Donoso and Cabello, 1978) or 16-18 months (Tortorelli, 1956; Montaldo, 1974) from pollination. Data herein obtained showed that gravity seed dispersal, which usually takes place at the end of the Summer (approx. 12-13 months after pollination), moved towards Autumn (14-15 months after pollination) in 2016, and then towards early Winter (16-18 months after pollination) in 2017.

Descriptor lists include the basic description of the traits, and the different classes of their expression (characterization) or how to measure the range of their variation (evaluation). Most of the descriptors for characterization and evaluation are species-specific, but should be preferentially evaluated under homogeneous growing conditions in order to obtain comparable data and to avoid potential environmental influence on the phenotype. In our study, in order to find the most appropriate descriptors able for distinguishing effectively between individual phenotypes without loss of discriminating power, repeated observations were made on all plant material available in the Pistoia's nursery district. It immediately became clear that there were no evident differences among the expression of growing characters of young potted seedlings (Fig. 7), despite the very high number of individuals within the various collections, whereas a high degree of between- and within-population variability was noted among older trees (Fig. 8). A first analysis of the available specimen clearly evidenced that plant habitus as well as various other vegetative traits consistently varied with plant age,

Fig. 7 - *Araucaria araucana* seedlings in Pistoia's nurseries (A: two-year-old seedlings; B: six-year-old seedlings).

being this species a long-lived and massive tree up to 50 m tall and 2 m in diameter and attaining maximum ages of at least 1300 years (Montaldo, 1974). Moreover, different climatic patterns and growing conditions, such as plant density and weed control, might have influenced plant growth and habitus as well.

The variability observed here is consistent with the outbreeding dioecious reproductive habit of this species and suggests that these populations should continue to be viable and able to respond to moderate levels of environmental change (Hadad and Roig Juñent, 2016). However, recent phytopathological analysis in Tuscany have revealed an increase in mortality, especially of female individuals, without a specific pathogen responsible (Rizzo, 2017, personal communication). The most widely accepted theory is that weaker plants, due to climate-related stress, are more vulnerable to damage caused by aspecific pathogens.

Fig. 8 - Habitus variability in *Araucaria araucana* trees grown in Pistoia's hinterland in a plain area.

A correct germplasm characterization should consider adult plants, being necessary the presence of flowers and fruits. However, as the aim of the present study is the phenotyping of young cultivated plants for sale, only trees sufficiently young for trade but at the beginning of sexual differentiation were suitable for characterization. This is why only twenty/twenty-five-year-old specimen belonging to a subset of 4 putative populations grown under similar environmental and agronomic conditions were considered. Plants over 25 year-old were discarded as well as plants grown in nearby locations.

With respect to the 39 descriptors detailed in the descriptor list, some very peculiar characters, i.e. insertion angle of the scales on the trunk (N. 8), uniformity of the apparent diameter on the primary and secondary branch (N. 19 and 20), catkin bending (N. 26), were individuated beside some of the most common traits, such as shape and density of canopy (N. 3 and 4), maximum length and width of the leaves (N. 22 and 23). Undoubtedly, one of the tree's most distinguishing feature is its scales, which are stiff, dark green and glossy with a spiny tip and completely cover each branch, closely overlapping each other. But we found surprisingly interesting the size together with the insertion angle of the scales on primary and secondary branches, resulting in evident dissimilar apparent diameters of branches. On the contrary, female inflorescence didn't show any distinctive feature among populations, therefore it was not taken into account as a descriptor. In fact, while the true flower represents the main distinctive character in Magnoliophyta, the Pinophyta flowers, that are extremely simple, are not particularly relevant for the description of the species.

In our study, morphological traits of *A. araucana* were observed, measured and documented for the first time under Tuscan growing condition. In particular, the research was developed using trees from putative populations growing in the Pistoia's nursery district. The comparison of the observed phenotypic characteristics showed a wide range of variability among and within the considered populations. The resulting data allowed to classify accessions, and to build a catalogue of specific descriptors with embedded biological information that is an essential step towards germplasm phenotyping (in particular new variety description), management or for direct use in agriculture. Limitations linked to the potential environmental influence on the phenotype are presented as well. Apart from this preliminary study, nothing is known of the patterns of morphological variation within this species. The development of this descriptor list will assist in the systematic and objective recording and exchange of information, which in turn will increase utilization of genetic resources along with a better screening and use of *A. araucana* biodiversity for breeding programs. In order to analyze relationships between individuals or groups of specimens within locally grown populations, the morphometric characterization of *A. araucana* trees is in progress based on the defined descriptor list and suitable statistical multivariate approaches. Genetic

analysis of the same populations are being performed and will be processed in order to validate the discriminating efficiency of the presented descriptor list.

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